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Prevalence and PCR-based follow-up of hemotropic mycoplasma infections in dogs in Switzerland

INAUGURAL-DISSERTATION
zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

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Zürich 2007

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1 Summary

Hemotropic mycoplasmas (hemoplasmas) have been reported in several mammalian species. In dogs, two species have been described: *Mycoplasma haemocanis* and ‚*Candidatus* Mycoplasma haematoparvum'. Infections can lead to hemolytic anemia, but the clinical importance of the two canine hemoplasma species needs to be further investigated. No data on the presence of canine hemoplasmas in Switzerland has been published, but the tick *Rhipicephalus sanguineus*, a proposed vector for canine hemoplasmas, is occasionally encountered in the canton Ticino. The aim of the present study was to investigate the importance of *M. haemocanis* and '*Candidatus* M. haematoparvum' in the Swiss dog population. Blood samples from 889 dogs were analyzed with two sensitive real-time PCR assays. For phylogenetic analyses, the 16S rRNA genes of four isolates were sequenced. Overall, hemoplasma infections were rarely detected in Swiss dogs: *M. haemocanis* was found in eight (0.9%) and '*Candidatus* M. haematoparvum' in three (0.3%) tested dogs. The sequenced isolates revealed ≥ 99.8% identity to published sequences. Only two infected dogs showed mild anemia. In one '*Candidatus* M. haematoparvum' and two *M. haemocanis* infected animals a chronic carrier status of three to 13 months was demonstrated. Interestingly, the majority of the infected dogs had paid visit to regions where *R. sanguineus* is frequently found. According to our results, canine hemoplasma infections play only a subordinate role in dogs in Switzerland.

2 Introduction

The presence of epierythrocytic bacteria in blood smears from dogs with anemia have been reported worldwide (2, 3, 11, 14, 23, 24, 33). The respective agent, *Haemobartonella canis,* has recently been reclassified within the group of hemotropic mycoplasmas (hemoplasmas) as *Mycoplasma haemocanis* (21). Phylogenetic studies based on the 16S rRNA and Rnase P genes revealed a close relationship of *M. haemocanis* to the feline pathogen *Mycoplasma haemofelis* (5, 21). Recently, a second canine hemoplasma was discovered in the United States of America (USA) in a splenectomized dog with hemic neoplasia and designated '*Candidatus* Mycoplasma haematoparvum' (27). The latter agent shows a close phylogenic relationship to the feline hemoplasma '*Candidatus* Mycoplasma haemominutum' (28).

Clinical cases of hemoplasmosis in dogs have occasionally been reported (2, 3, 7, 11, 14, 19, 23, 33). Co-factors such as splenectomy (3, 7, 14, 19, 33), immunosuppression (2) or concurrent infections (11, 33) seem to play a role in the pathogenesis of canine hemoplasmosis. A chronic carrier status may be present in healthy dogs (3, 12). Of note, most of the so far published studies relied upon cytological identification of the organisms on blood smears (2, 3, 11, 19, 23, 24, 33), a method with a low diagnostic sensitivity and specificity (15). The recent development of PCR-based assays to diagnose canine hemoplasmas allows a more sensitive detection of these agents and the differentiation of the two described species (16). So far, only one study used real-time PCR-based methods to investigate the prevalence of canine hemoplasmosis in a sample of 460 dogs from Southern France (16): overall, 15.4% of the dogs tested PCR positive for canine hemoplasmas. Noteworthy, *R. sanguineus*, a proposed vector for canine hemoplasmas (26) is commonly encountered in Southern France (10). Recently, higher infection rates have been reported in kennel-raised dogs from Western Europe, Eastern Europe and North America (15). It was hypothesized that the possibility of a

transplacental transmission (18) would perpetuate an infection, once established, in kennel-raised dogs (15). However, the transmission routes of canine hemoplasmas are still unknown.

This study aimed I) to investigate for the first time the presence of hemoplasma infections in dogs in Switzerland using sensitive real-time PCR assays, II) to follow hemoplasma-infected dogs over time and III) to molecularly characterize the hemoplasma isolates.

3 Materials and Methods

3.1 Sample collection

EDTA-anticoagulated blood samples from 889 Swiss dogs were collected throughout a one year period starting from December 2005. All but one dog were presented for various reasons at the Clinic for Small Animals, Vetsuisse Faculty, University of Zurich, Switzerland: they included 882 sick dogs from which blood samples had been taken for diagnostic purposes and six healthy blood donors. The blood sample from one sick dog was sent in by a practicing veterinarian for diagnostic purposes. In addition, seven follow-up blood samples from three hemoplasma PCR-positive dogs were available.

3.2 Hematological analysis

Packed cell volume (PCV) values determined on a Cell-Dyn 3500 (Abbott, Baar, Switzerland) were available from 875 dogs. PCV values between 42% and 55% (5% and 95% quantiles, reference range of the Clinical Laboratory, Zurich, Switzerland) were considered to be within the normal range; anemia was defined as a PCV value below 42%.

3.3 Medical records

Information about gender and age of all dogs included in this study were obtained from the Clinic for Small Animals of the University of Zurich, Switzerland. Complete medical records were only recorded for hemoplasma PCR-positive dogs. The latter data included breed, place of domicile, clinical diagnosis, treatment and travel history (to foreign countries or Southern Switzerland, canton Ticino). The presence of an intact spleen was confirmed by data from X-ray or ultrasonographic investigations.

3.4 TNA extraction

Total nucleic acids (TNA) were extracted from 200 µl (155 initial and one follow-up sample) or from 100 µl (734 initial and six follow-up samples) EDTA-anticoagulated blood using the MagNaPure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Rotkreuz, Switzerland). Negative controls consisting of 200 µl phosphate buffered saline were prepared with each batch of 15 samples. Extracted samples were stored at -80°C until use.

3.5 Quantitative real-time PCR assays and standards

All samples were analyzed by real-time PCR for the presence of canine hemoplasmas. For the detection of *M. haemocanis* a published *M. haemofelis* real-time PCR assay was used; due to high sequence homology, the latter assay amplifies both hemoplasmas (30). A modified ‚*Candidatus* M. haemominutum' real-time PCR assay (30) was used for the detection of ‚*Candidatus* M. haematoparvum' with the forward primer adapted as follows: 5'-GAA AGT CTG ATG GAG CAA TAC CAC-3'. To evaluate the sensitivity of the latter assay, 77 samples were additionally analyzed with a published real-time PCR assay for ‚*Candidatus* M. haematoparvum' (16).

All real-time PCR assays were performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Rotkreuz, Switzerland). In each run, the amplification buffer contained dUTP for the use with uracil-N-glycosylase to prevent carryover of PCR amplicons and water was used as a negative control.

For absolute quantification, plasmids containing the cloned 16S rRNA gene of '*Candidatus* M. haematoparvum' were generated (see below). Purified DNA was linearized by restriction digestion with *Not*I (Promega, Catalys AG, Wallisellen, Switzerland) and the copy number was calculated by both spectrophotometer (GeneQuantTM, Amersham-Parmacia Biotech, Otelfingen,

Switzerland) and agarose gel electrophoresis (Syngene, Gene Tools software, Syngene, Cambridge, United Kingdom). The ‚*Candidatus* M. haematoparvum' DNA was serially tenfold diluted in a solution of 30 µg/ml of salmon sperm DNA (Invitrogen, Basel, Switzerland) (31). For *M. haemocanis* quantification, a published standard based on the 16S rRNA gene of *M. haemofelis* was used (30).

3.6 Sequencing of 16S rRNA genes

The near complete 16S rRNA gene from three *M. haemocanis* isolates (dog 201, 1725 and 1769; Table 1) and one '*Candidatus* M. haematoparvum' isolate (dog 252; Table 2) was sequenced. For both agents, primers were designed (*M. haemocanis* forward primer 5'-TCG AAC GGA CCT TGG TTT CG-3' and reverse primer 5'-CAA ATA AAT GTA TTT TTA AAT GCC CAC-3', '*Candidatus* M. haematoparvum' forward primer 5'-GCT CAG GAT TAA TGC TGG TGG-3' and reverse primer 5'-GGG CGG TGT GTA CAA GAC CT-3') that amplify a 1330 bp and 1354 bp fragment of the gene, respectively. The reaction mixture contained 5 µl of 5 X HF PCR buffer (Finnzymes, BioConcept, Allschwil, Switzerland), 800 nM each primer, 200 nM of each deoxynucleoside triphosphate (Sigma-Aldrich, Buchs, Switzerland), 1 U Phusion High-Fidelity DNA Polymerase (Finnzymes) and 5 µl template TNA made up to 25 µl with water. The program comprised 98°C for 3 min, 35 cycles of 98°C for 10 s, 60.8°C (*M. haemocanis*) or 63.2°C ('*Candidatus* M. haematoparvum') for 30 s, 72°C for 1 min, and a final elongation at 72°C for 10 min. A-tailing was performed in a reaction mixture comprising 8 µl of the PCR product, 1.2 µl of 10 X PCR buffer, 200 nM dATP and 1 U of Taq DNA Polymerase (all reagents from Sigma-Aldrich) filled up with water to a final volume of 12 µl. The reaction was performed at 72°C for 15 min. The TOPO TA Cloning Kit for Sequencing containing the pCR 4–TOPO vector (Invitrogen) was used for cloning and plasmid DNA was purified using the QIAprep Spin Miniprep Kit (Qiagen,

Hombrechtikon, Switzerland). Sequencing was performed with M13 forward and reverse primers and an internal primer (5'-AGC AAT ACC ATG TGA ACG ATG AA-3') using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) under standard cycling conditions. Products were purified with SigmaSpin Post-Reaction Clean-Up Columns (Sigma-Aldrich) and analyzed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). For sequence comparison, the GCG Wisconsin Package (Accelrys GmbH, Munich, Germany) was used. A phylogenetic tree was constructed with the neighbor-joining method from a distance matrix corrected for nucleotide substitutions by the Kimura two-parameter model (17).

3.7 Statistics

Categorical variables (gender, presence of anemia and infection) were compared between groups using the Chi^2 Test (6). Age was compared between PCR-positive and -negative dogs using the Mann-Whitney U Test (6).

3.8 Nucleotide sequence accession numbers

The partial 16S rRNA gene nucleotide sequences have been submitted to GenBank (*M. haemocanis*: EF416566 - EF416568; '*Candidatus* M. haematoparvum': EF416569).

4 Results

4.1 Real-time PCR assays

All extraction and PCR controls tested PCR negative. The '*Candidatus* M. haematoparvum' assay was able to detect 1 copy/reaction of '*Candidatus* M. haematoparvum' DNA (10/10 reactions positive). All 77 samples that were tested in parallel with two different real-time PCR assays for '*Candidatus* M. haematoparvum' revealed identical results (one out of 77 samples positive).

4.2 Sample prevalence of canine hemoplasmas in the Swiss dog population

M. haemocanis DNA was detected in eight (0.9%) and '*Candidatus* M. haematoparvum' DNA in three (0.3%) out of 889 Swiss dogs. No dog was found to be dually infected with both canine hemoplasmas. The samples from the six healthy blood donors included in the study were found to be PCR negative.

4.3 Hematology analysis

Out of the 864 PCR-negative dogs with hematological data, 295 (34.1%; 143 female and 152 male dogs) were considered anemic, 547 (63.3%; 277 female and 270 male dogs) had PCV values within the reference range, and 22 dogs (2.5%; 14 female and 8 male dogs) had values above the reference range. In the eleven hemoplasma-infected dogs, the PCV values of nine animals initially were within the reference range (81.8%); one infected dog showed a slight anemia (PCV 40%). One infected greyhound had a PCV value of 62%. The latter value can be judged as normal, because this breed is known to show physiologically elevated PCV values. No significant association between the occurrence of canine hemoplasma infection and anemia was found.

4.4 Hemoplasma blood loads

All samples from the eleven PCR-positive dogs underwent quantitative real-time PCR assays for the assessment of hemoplasma loads. Seven *M. haemocanis* PCR-positive dogs had loads ranging from 33 to 5.2 x 10^5 DNA copies per ml blood. One of these *M. haemocanis* PCR-positive dogs tested positive in only one out of six PCR samples (33 DNA copies per ml blood). The '*Candidatus* M. haematoparvum' blood loads of two PCR-positive dogs were 5.8 x 10^2 and 2.1 x 10^3 DNA copies per ml blood, respectively. The load of one *M. haemocanis* and one '*Candidatus* M. haematoparvum' PCR-positive dog could not be quantified because the samples repeatedly tested real-time PCR negative when rerun for absolute quantification. Thus, the loads in these animals were assumed to be < 33 DNA copies per ml blood.

4.5 Characteristics of hemoplasma PCR-positive dogs

Five hemoplasma infected dogs were of male gender, six were females (gender of the PCR-negative dogs: 437 males, 441 females); no significant association between gender and presence of a canine hemoplasma infection was found. The age of the infected dogs ranged from three to 13 years (median age: 8 years; 5% quantile: 4 years, 95% quantile: 12.5 years); for the PCR-negative dogs the age ranged from below one to 18 years (median age: 8 years; 5% quantile: 1 year, 95% quantile: 14 years). No significant difference in age was found between the PCR-negative and -positive dogs. Eight hemoplasma-infected dogs were pure bred; three animals were crossbred (Tables 1 and 2).

Ten hemoplasma-infected dogs had their place of domicile north of the Alps, one lived in Southern Switzerland (canton Ticino). At least two hemoplasma-infected dogs sojourned in Southern Switzerland (Table 1). Ten out of eleven infected dogs (90.9%) have not spent their entire life in Switzerland. Travel

histories are listed in Tables 1 and 2. Three dogs were imported from Italy and two dogs were adopted from Mexico and Spain, respectively. Travel history was incomplete in dog 954 (Table 1).

The hemoplasma infected dogs were presented at the clinics for diverse reasons. Four infected dogs were diagnosed with tumors (Tables 1 and 2). Two dogs showed lacerations due to fighting and one animal had a degloving limb injury (hit by car). Gastrointestinal, cardiac and muscular diseases were diagnosed in four animals (Tables 1 and 2). Eight of the PCR-positive dogs have not undergone splenectomy. No data from radiology or ultrasonography was available from the remaining three infected dogs. Two infected dogs were iatrogenic immunosuppressed by prednisolone and lomustine, respectively (see below).

4.6 Follow-up of hemoplasma-infected dogs

For three hemoplasma PCR-positive dogs, a total of seven follow-up data samples were available; all seven samples yielded PCR-positive results (Fig. 1). The '*Candidatus* M. haematoparvum' infected dog 252 remained PCR positive for a 400 day follow-up period (Fig. 1A). This dog underwent cytostatic chemotherapy with lomustine (60-70 mg/m^2 body surface area every three week). The dog received four applications, the last one at the time of the first blood sample collection for the present study (Fig. 1A).

Two *M. haemocanis* PCR-positive dogs remained positive for a period of 100 days and 270 days (Fig. 1B and C). The *M. haemocanis* infected dog 1725 (Fig. 1B) was presented nine months after initial presentation in a very poor general condition (suspicion diagnosis: metastasizing cancer) and was euthanized. The PCV value of this dog had decreased from 42% at the first visit to 34% at this second appointment. The second *M. haemocanis* infected dog with follow-up data (dog 201, Fig. 1C) was treated with an immunosuppressive dosage of prednisolone up to the day of the second blood

investigation by PCR, the medication was thenceforward reduced to an anti-inflammatory dosage (Fig. 1C). Only moderate fluctuations of the blood loads were seen in the *M. haemocanis* and the ‚*Candidatus* M. haematoparvum' follow-up samples with a maximal decrease by a factor of 40 within one week in the *M. haemocanis* infected dog 201 (Fig. 1C).

4.7 Sequencing of 16S rRNA genes

To confirm the PCR results and to evaluate sequence variations, the 16S rRNA genes of four canine hemoplasma isolates were almost completely sequenced. The data revealed 99.9% to 100% identity of the three *M. haemocanis* isolates with a published *M. haemocanis* sequence (AF407208) and 99.8% identity of the ‚*Candidatus* M. haematoparvum' isolate with a published sequence of ‚*Candidatus* M. haematoparvum' (AY383241). Phylogenetic analyses revealed that the Swiss ‚*Candidatus* M. haematoparvum' isolate clustered together with an isolate from the USA (Fig. 2). All three *M. haemocanis* isolates from Switzerland were localized within the same subcluster as were all *M. haemocanis* isolates included in the phylogenetic tree (Fig. 2).

5 Discussion

This is the first study to investigate the occurrence of canine hemoplasma infections in dogs in Switzerland. In addition, we document for the first time PCR-based follow-ups for the two canine hemoplasma species.

Hemotropic mycoplasma infections were rarely detected in Swiss pet dogs (1.2%), suggesting that these agents play only a marginal role in dogs in Switzerland. Recently, a real-time PCR-based study in 460 dogs from Southern France detected 15 (3.3%) *M. haemocanis* and 44 (9.6%) '*Candidatus* M. haematoparvum' singly infected dogs and 12 (2.6%) dogs co-infected with both hemoplasmas (16). A study based on conventional PCR in free-roaming dogs in Sudan found even higher hemoplasma infection rates (13). Interestingly, more than half of the latter dogs were found infested with the tick *R. sanguineus*, a proposed vector for canine hemoplasmas (26). In contrast to Southern France and Sudan, *R. sanguineus* is not indigenous to Switzerland; it is only occasionally encountered in the southern part of the country (canton Ticino) (4) whereas in Northern Switzerland, the climate is too cold for *Rhipicephalus* ticks to permanently establish. Most ticks in Switzerland belong to the genus *Ixodes*, but no feline and canine hemoplasma DNA was recently detected by real-time PCR in almost 2,000 *Ixodes* ticks collected from vegetation in Northern Switzerland (32). Thus, we hypothesize that the low prevalence of canine hemoplasma infections in Switzerland may be due to the lack of appropriate arthropod vectors and that canine hemoplasmosis plays predominantly a role in countries where dogs are commonly exposed to *Rhipicephalus* ticks. This is further supported by the finding that the majority of the infected dogs in the present study had visited countries where *R. sanguineus* is permanently established, such as Italy, Spain, France, Serbia, the USA and Mexico (1, 8-10, 22, 25); these dogs could have acquired the hemoplasma infection abroad. Three infected dogs in the present study spent time or lived in the canton Ticino, where *R. sanguineus* is occasionally

encountered. A recent study failed to detect canine hemoplasmas in *Rhipicephalus* ticks collected from pet animals in the canton Ticino (32). However, this could be due to the low sample size under investigation (23 *R. sanguineus*, 44 *Rhipicephalus sp.*). Future studies should therefore be conducted to investigate different arthropod vectors and other potential ways of transmission in more depth to address their role in the epizoology of canine hemoplasma infections.

Recently, hemotropic mycoplasma infection in cats was found to be associated with male gender, old age and sometimes anemia (29, 30). In contrast, but in accordance with the real-time PCR based investigations in French dogs (16), we found no association of canine hemotropic mycoplasma infection with gender and age in the dogs. However, due to the small number of infected dogs resulting in a low statistical power these associations might have been missed.

No hemoplasma-infected dogs in the present study exhibited clinical signs attributable to hemoplasmosis and PCV values of all infected dogs were found to be within or close to the reference range. This finding is in accordance with previous publications which suggested that co-factors, such as splenectomy or severe immunosuppression, are involved in the pathogenesis of canine hemoplasmosis (2, 3, 7, 14, 19, 20, 33). In view of the limited pathogenic potential in immunocompetent hosts and the low hemoplasma prevalence reported in this study, routine screening of blood donors for canine hemoplasmas seems not of utmost importance in dogs in Switzerland. The six blood donors that had been included in the present study tested all negative for canine hemoplasmas. Nonetheless, the investigation of blood donors by sensitive PCR-based methods should be considered in countries with high infection rates or if the recipient dog is splenectomized or severely immunocompromised.

It has been suspected that hemoplasma-infected dogs remain chronic carriers (3, 20). So far, the confirmation of this assumption was hampered by the low sensitivity of microscopy-based diagnostics. In one case a chronic carrier status was confirmed in a experimentally infected dog by the occurrence of organisms in blood smears upon splenectomy one year after inoculation (3). By means of sensitive real-time PCR-based diagnostics, we now followed chronic carrier dogs for three to 13 months: all follow-up samples of a '*Candidatus* M. haematoparvum' and two *M. haemocanis* infected dogs tested PCR positive.

The hemoplasma blood loads in the present study were rather low and could not be exactly determined in two hemoplasma PCR-positive dogs, although the two applied PCR assays were shown to be highly sensitive (30). More PCR reactions would have been necessary to accurately quantify the blood loads in these two dogs. It may be speculated that other dogs might have had similarly low hemoplasma loads and that thus some hemoplasma-infected dogs were not detected in the present study. However, we assume that hemoplasma infections with blood loads close to the detection limit are not of great clinical relevance.

In summary, canine hemoplasma infections were rarely detected in dogs in Switzerland. Phylogenetic analysis of the 16S rRNA gene revealed a high identity (>99%) of *M. haemocanis* and '*Candidatus* M. haematoparvum' isolates from dogs in Switzerland to previously published sequences from dogs in other countries. The use of sensitive PCR-based methods represent an essential prerequisite to investigate in more depth the pathogenesis and possible transmission routes of canine hemoplasmas and the role of chronic carrier dogs in the epizoology of these agents.

6 Figures and Tables

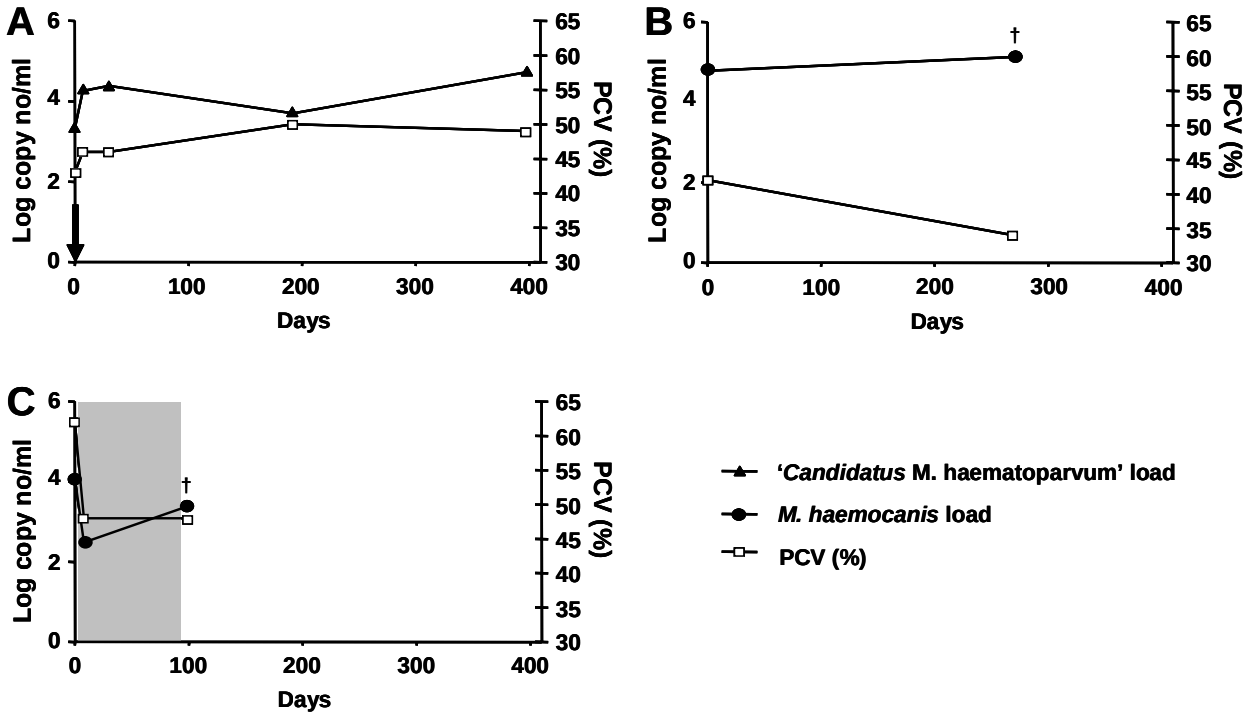

FIGURE 1. Follow-up of hemoplasma infected dogs.

Course of '*Candidatus* M. haematoparvum' (A) and *M. haemocanis* (B-C), infections. The loads are presented as log DNA copy number per ml of blood (left y-axis); the PCV is given in % (right y-axis). A) dog 252, B) dog 1725 and C) dog 201. The arrow (A) indicates the end of antineoplastic chemotherapy with lomustine in dog 252. The period of prednisolone administration in dog 201 (C) is indicated by a gray area. † dog was subsequently euthanized for reasons unrelated to canine hemoplasmosis.

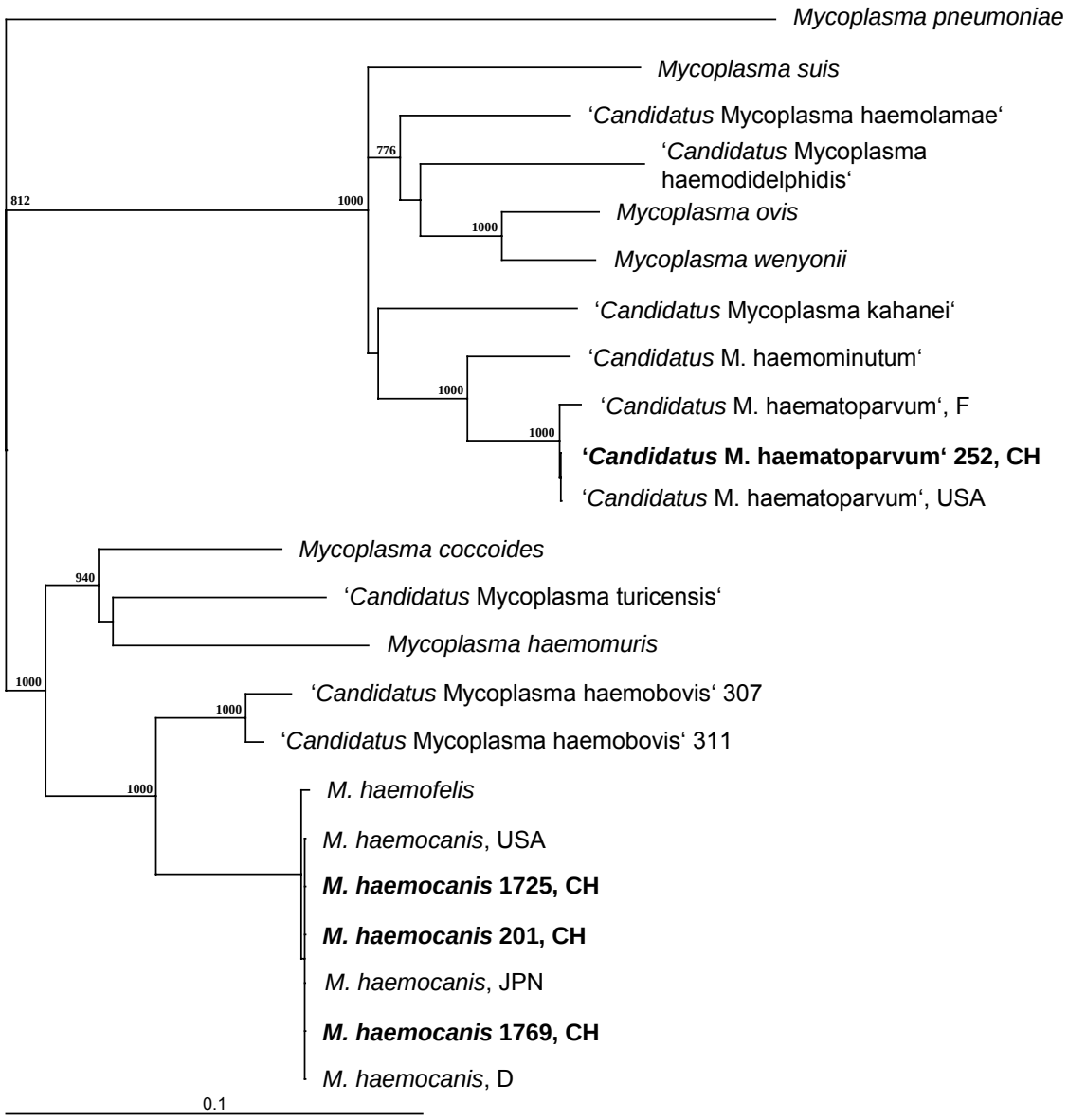

FIGURE 2. Phylogenetic tree.

Phylogenetic tree demonstrating the relationship of the Swiss canine hemoplasma isolates to other hemoplasma species using the neighbor joining method. *Mycoplasma suis* (AF029394), '*Candidatus* Mycoplasma haemolamae' (AF306346), '*Candidatus* Mycoplasma haemodidelphidis'

(AF178676), *Mycoplasma ovis* (AF338268), *Mycoplasma wenyonii* (DQ641256), '*Candidatus* Mycoplasma kahanei' (AF338269), '*Candidatus* M. haemominutum' (DQ157148), '*Candidatus* M. haematoparvum' (dog 252, Switzerland, EF416569; France, AY532390; USA, AY383241), *Mycoplasma coccoides* (AY171918), '*Candidatus* Mycoplasma turicensis' (DQ157150), *Mycoplasma haemomuris* (U82963), '*Candidatus* Mycoplasma haemobovis' (Switzerland, M.L. Meli, in preparation), *M. haemofelis* (DQ157157) and *M. haemocanis* (dog 201, Switzerland, EF416566; dog 1725, Switzerland, EF416568; dog 1769, Switzerland, EF416567; USA, AF407208; Japan, AY529641; Germany, AY150973) are shown. *Mycoplasma pneumoniae* (U00089) was used as an out-group. The numbers at the nodes were generated from 1,000 bootstrap resamplings; values of <700 are not shown.

TABLE 1. Dog identity, breed, gender, age, PCV, clinical diagnosis and recorded travel history of *M. haemocanis* PCR-positive dogs.

No.[1]	Breed	Gender[2]	Age (years)	PCV[3] (%)	Clinical diagnosis	Travel history (countries visited)	Travel history (to canton Ticino)
201	Greyhound†	fn	9	62	Protein loosing enteropathy	Spain	No
551	Bloodhound	fn	7	55	Lipoma	No	Yes
677	Lagotto Romagnolo†	mc	10	45	Brain tumor	Italy	No
954	Alaskan Malamute†	fn	6	40	Myositis	Germany[4]	Unknown[4]
1619	Rottweiler	fn	7	45	Mast cell tumor, benign spleen nodule	France	Yes
1725	Rottweiler†	m	5	42	Bite wound	Serbia	No
1769	Dachshund	m	13	47	Mitral valve endocardiosis	Serbia, Austria	No
1857	Crossbreed	m	6	54	Degloving limb injury	Italy	Yes

[1] No. = Dog identity number; [2] m = male, f = female, c = castrated, n = neutered; [3] Reference range: 42 - 55% (5 to 95% quantile); [4] data incomplete because dog 954 was adopted at the age of 4 years;

† dogs were euthanized or died subsequently for reasons unrelated to canine hemoplasmosis.

TABLE 2. Dog identity, breed, gender, age, PCV, clinical diagnosis and recorded travel history of '*Candidatus* M. haematoparvum' PCR-positive dogs.

No.[1]	**Breed**	**Gender**[2]	**Age (years)**	**PCV**[3] **(%)**	**Clinical diagnosis**	**Travel history (countries visited)**	**Travel history (to canton Ticino)**
252	Crossbreed	mc	12	43	Mast cell tumor	Mexico, USA, Italy	No
519	Maltese	f	3	49	Bite wound	Italy	No
1756	Crossbreed	fn	10	47	Gastroenteritis	Spain	No

[1] No. = Dog identity number; [2] m = male, f = female, c = castrated, n = neutered; [3] Reference range: 42 - 55% (5 to 95% quantile).

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8 Danksagung

An dieser Stelle möchte ich mich ganz herzlich bei allen bedanken, die zum Gelingen dieser Arbeit beigetragen haben. Ein besonderes Dankeschön geht an Prof. Dr. Regina Hofmann-Lehmann für die super Betreuung und für ihr tolles Engagement. Sie war immer für mich da!
Ganz herzlichen Dank an Dr. Barbara Willi. Sie hat mich in die Welt der Haemoplasmen eingeführt und ist mir während meiner Zeit im Labor immer tatkräftig zur Seite gestanden. Zudem denke ich gerne an all die schönen Stunden, die wir auch ausserhalb des Labors zusammen verbrachten.
Mein Dank geht auch an Prof. Dr. Hans Lutz für seine Unterstützung und für sein ansteckendes Forschungsinteresse.
Ein herzliches Dankeschön geht an die Frauen vom Routinelabor, welche mir oft die Hundeblutproben zum Sammeln beiseite stellten und die mir im Labor immer wieder spannende Blutausstriche zeigten.
Des Weiteren möchte ich mich beim ganzen Laborteam für die super Unterstützung und für die gute Zeit bedanken.

Weiter gilt mein Dank dem ganzen Team der Veterinärbakteriologie der Vetsuisse Fakultät Zürich. Während der Zeit meiner Dissertation arbeiteten wir gemeinsam an einem weiteren Haemoplasmenprojekt und ich bedanke mich insbesondere bei Dr. Katharina Hölzle ganz herzlich für die tolle Zusammenarbeit.

Zum Schluss möchte ich mich ganz besonders bedanken bei meinem Freund Raphi, meinem Mami, meiner Schwester und meinem Bruder und bei all meinen Freundinnen und Freunden, die immer für mich da sind, mich unterstützen und ohne die meine Welt nur halb so farbig wäre. Danke!

Lebenslauf

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7. Juni 2007